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Discovery and design of cyclic peptides as dengue virus inhibitors through structure-based molecular docking

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ABSTRACT

Objective: To find potential peptide inhibitors against the NS2B/NS3 protease of DENV which in turn, can inhibit the viral replication inside host cell. **Methods:** Cyclic peptides were designed having combination of positively charged amino acids using ChemSketch software and were converted to 3D structures. DENV NS3 protein structure was retrieved from Protein Data Bank (PDB) using PDB Id: 2FOM. DENV NS3 and cylic peptides were docked using MOE software after structural optimization. **Results:** Through molecular docking it was revealed that most of the peptides bound deeply in the binding pocket of DENV NS2B/NS3 protease an had interactions with catalytic triad. Peptide 2 successfully blocked the catalytic triad of NS2B/NS3 protease. Peptide 1, ,4 and 6 also had potential interactions with active residues of the NS2B/NS3 protease while all other peptides were in close contact with the active sites of NS2B/NS3 protease thus, these peptides can serve as a potential drug candidate to stop viral replication. **Conclusions:** Thus, it can be concluded from the study that these peptides could serve as important inhibitors to inhibit the viral replication and need further in-vitro investigations to confirm their efficacy.

1. Introduction

Dengue infection has become a worldwide health issue and approximately 2.5 billion people are being affected with this dreadful disease[1]. During recent years, it has become prevalent in more than 100 countries and according to an estimation of WHO, about 50-100 million people are being caught with this disease[2]. Dengue virus (DENV) belongs to Flaviviradae family and has four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4)[3]. Two mosquito known as *Aedes aegypti* and *Aedes albopictus* serves as vector in transimission of DENV infection among human[4]. The regions where this infection has become a problem includes Asia, Central and South America and Africa[5,6]. DENV causes two types of infections known as primary and secondary infections. Primary infection includes acute febrile fever known as dengue fever. Dengue fever can be cleared through complex immune responses in about 7 days of infection. Secondary infection is a more serious and severe form of infection which includes dengue hemorrhagic fever or dengue shock syndrome[7].

The genome of DENV is 11 kbp and encodes a polyprotein which is proteolyticaly cleaved into 10 viral proteins including three structural and seven non-structural (NS) proteins. The order of these viral proteins is capsid, premembrane, envelope protein, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The structural proteins are responsible for the structure of virus and non-structural proteins are necessary for the viral replication[8].

According to studies, it has been found that NS3 protein is most important non-structural protein having a trypsinline serine protease domain at the N terminal region. This region is known as NS3pro and its activity depends on its interaction with cofactor (NS2B). Together these two forms a complex named NS2B/NS3pro complex. This complex is important because of its ability to cleave at different parts of viral proteins. Any changes/disruption in functional activities of this region can lead to the inhibition of viral replication. Thus, NS2B/NS3 complex is considered as an important target to screen and evaluate effects of different

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drug candidates[9]. Recent computational techniques have opened new doors to drug development studies. During recent years, researches have been conducted to understand the effect of disulphide cyclic peptides against NS2B/NS3 complex to inhibit DENV infection. These studies have provided interesting results of cyclic peptides and shown the potential of these peptides in DENV inhibition[10,11].

Molecular docking is a method of binding orientation prediction of small molecules with their protein targets. These computational techniques provide information on binding activity and binding affinity of the molecules against their target protein. Thus, molecular docking is considered as important technique in drug designing and screening of novel antiviral compounds against challenging diseases[12]. This study was designed to screen novel peptides as DENV inhibitors to target the hydrophobic pockets of DENV NS2B/NS3 protease, using computational docking techniques.

2. Material and methods

Disulphide cyclic peptides have been docked against DENV NS2B/NS3 protease using the MOE (Molecular Operating Environment) software package.

2.1. Receptor refinement

Three-dimensional (3D) structure of the DENV-2 NS2B/NS3 protease was retrieved from the database of Protein Data Bank (PDB) using PDB ID: 2FOM. Optimization of the structure was done by adding hydrogens using the MOE software[13]. To optimize the structure, H_2O molecules were removed from the structure and 3D protonation was done to change the state into ionization level. Moreover, energy minimization was done using parameters such as force field: MMFF94X+Solvation, gradient: 0.05, and chiral constraint: current geometry. This minimized structure was used for docking studies.

2.2. Peptide designing

Positively charged amino acid residues of substrates are likely to interact with the specific binding site of the DENV NS2/NS3 protease. According to a study on NS3, P1 position of the enzyme's binding site has likeness of interaction with basic amino acid residues (Arg/Lys), whereas the preference for the P2 is Arg > Thr > Gln/Asn/Lys and for P3 is Lys > Arg > Asn[14]. Tri and tetra peptides were designed using combination of these amino acid residues. Seven peptides (four tri and three tetra) were designed to investigate their potential against DENV NS2/NS3 protease. Furthermore, disulphide bridges were created by adding cysteine residues at both ends to convert these peptides into cyclic peptide. The designed peptides were then converted into their respective 3D structures using Chem Sketch software[15].

2.3. Peptide refinement

All the designed peptides were optimized by adding hydrogens using MOE software. Energy of the peptides was minimized using parameters such as gradient: 0.05, Force Field: MMFF94X, Chiral Constrzaint and Current Geometry. All these peptides were saved in mdb database which was further used for docking studies.

2.4. Molecular docking

The docking algorithm of the MOE software was used to dock these peptides with the catalytic triad (His 51, Asp 75, Ser 135) of DENV NS2/NS3 protease. The parameters were set as Re-scoring function: London dG, placement: triangle matcher, Retain: 5, Refinement: Force field, and Re-scoring 2: London dG. Docking program of MOE provides correct conformation (with the rotation of bonds, structure of molecule is not rigid) of the ligand so as to obtain minimum energy structure. Top conformation for each peptide was selected on the basis of S score and were further evaluated to study the hydrogen bonding/π-π interactions.

3. Results

The 3D X-ray crystallography structure of DENV NS2/NS3 protease was retrieved using PDB ID: 2FOM which was having resolution of 1.50 Å. All disulphide cyclic peptides were docked with the catalytic triad of NS2B/NS3 Protease.

3.1. Molecular docking

MOE docking program provided five conformations for each peptide. All the conformations were sorted according to S score and top tanking conformation with minimum S score were further analyzed. Peptide 2 was ranked as top conformation followed by peptide 1. All other conformations were having score close to each other (Table 1). Top conformation for each peptide was analyzed to find hydrogen bonding/π-π interactions.

3.2. Interaction analysis

Besides having minimum S score, peptide 2 (NRK) also had interactions with all the three residues (His 51, Asp 75 and Ser 135) of catalytic triad and thus, it can be concluded that this peptide could serve as a potential drug candidate against NS2B/NS3 protease. Moreover, peptide 1 (RKG) which was ranked second had potential interactions with Asp 75 and Ser 135 of catalytic triad. Peptide 4 (KRR) and peptide 6 (GNRK) had strong interactions with Ser 135 of catalytic triad. All other peptides (RGK, GRKR, and KKRR) didn't have any potential interaction with catalytic triad but had hydrophobic contact with active residues of catalytic triad.

Table 1
Peptide interactions with DENV NS2B/NS3 protease.

Sr. No.	Peptide	S score	RMSD	Interacting residues (Hydrogen bonding)	Close contact residues
1	Cys-RKG-Cys	-10.106 980	3.335 608	Arg54, Asp75, Glu101,Ser135, Asn152, Gly153	Trp50, His51, Leu 128, Pro132, Tyr 150
2	Cys-NRK- Cys	-11.129 510	2.531 304	His51, Asp75, Glu101, Asp129, Phe130, Ser135, Tyr150, Asn152, Gly153, Tyr161	Leu128, Pro132
3	Cys-RGK-Cys	-7.159 595	2.026 983	Phe130, Asn152, Gly153	His51, Asp75, Leu128, Pro132, Ser135, Tyr150, Gly151
4	Cys-KRR-Cys	-8.459 515	2.113 997	Trp50, Ser135, Gly151, Gly153	Hist51, Arg54, Asp75, Tyr150, Asn152, Tyr161
5	Cys-GRKR-Cys	-8.357 669	2.004 721	Leu128, Phe130, Gly153	His51, Ser127, Asp129, Ser131, Pro132, Ser135, Tyr150, Gly151, Tyr161
6	Cys-GNRK-Cys	-7.681 766	1.846 913	Phe130, Ser135, Gly151, Asn152, Gly153	His51, Asp75, Leu128, Pro132, Tyr150, Tyr161
7	Cys-KKRR-Cys	-8.353 346	2.254 074	Arg54, Leu128, Phe130, Asn152, Gly153	His51, Pro132, Ser135, Gly151, Val154, Tyr161

Interacting residues of the NS2B/NS3 protease are shown in Table 1. Interactions between receptor and ligands are shown in Figure 1. Binding mode of ligands with receptor protein is shown in Figure 2.

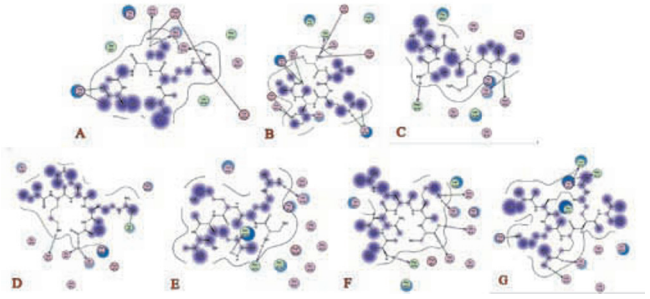

Figure 1. Binding interactions of peptides with active residues of DENV NS2B/NS3 protease.

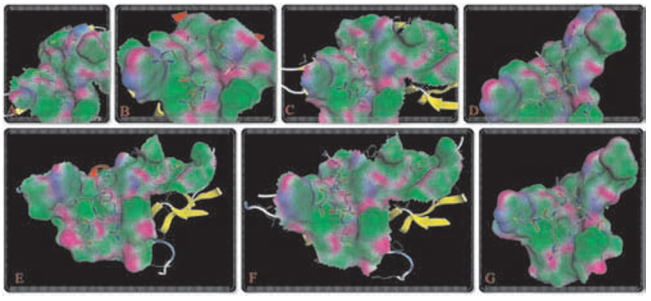

Figure 2. Docked complexes of peptides with the binding pocket of DENV NS2B/NS3 protease.

4. Discussion

Dengue is a dreadful disease and requires immediate attention to develop novel inhibitory compounds that could work against it. Genome of dengue encodes a single polyprotein precursor which is cleaved into 10 viral proteins[16]. The processing and cleavage of polyprotein precursor require signal peptidase and NS3 serine protease which requires a cofactor named NS2B[17]. DENV has four distinct serotypes[18] but the binding site of NS2B/NS3 has the same substrate specificities; thus, any inhibitor against the binding pocket of NS2/NS3 protease could work against all the serotypes[14]. DENV NS3 protease like other flaviviruses has been considered as an important drug target. The active site that is responsible for the activity is a catalytic triad (His 51, Asp 75 and Ser 135). As catalytic triad is important in viral replication; therefore, targeting it may block the viral replication[19].

During recent years, computational techniques have enabled researchers to evaluate the binding affininty of compounds before synthesizing and evaluating them in lab. These computational techniques have provided molecular insights of important viral genes and thus has contributed hugely in developing novel inhibitory compounds. Molecular docking is a techniques used to find the binding orientation of the small molecules against their targets. This technique is considered important in identification of novel inhibitory compounds against dreadful and challenging diseases[12]. The current study focuses on the designing and molecular docking of the tri and tetra peptides against NS2B/NS3 protease.

We investigated the potential of four tri and three tetra peptides against DENV NS2B/NS3 protease. The seven peptides that we designed were stabilized by adding S-S disulphide bridge which conveted them into cyclic form. The cyclic form of the peptides can lower the hydrogen interaction with solvent (H_2O) and can increase hydrophobic interactions within peptide which in turn can raise the stability and total entropy of the peptide[19]. In the present study, tri and tetra peptides were docked with the catalytic triad of NS2/NS3 protease to find their affinity as DENV inhibitors. After docking, only top conformations were selected on the basis of S score. The negative and low score for any ligand shows favorable interactions between ligand and the receptor protein. Our results showed that peptide 2 had minimum S score and also had strong interactions with

the active site residues of catalytic triad. All the peptides showed low energy and thus formed a stable ligand-receptor complex.

Through our study it was found that peptide 2 successfully blocked NS2B/NS3 protease and may serve as an important dug candidate against NS3 prtoease. Moreover, it was found that peptide 1, 4 and 6 had potential interactions and could serve as important drug candidates to block DENV infection. Although we have identified some potential peptides against NS2/NS3 protease but further study needs to be conducted on the absorption, distribution, metabolism, excretion, and toxicity of peptides proposed as a drug.

Dengue is a worldwide health problem and to date, no vaccine has been developed to target this challenging disease. Improved and novel strategies are required to develop drug candidates that can target DENV. Current study focuses on the designing and docking of cyclic peptides against NS2/NS3 protease. This study has revealed potential binding peptide (NRK) which interacts with the active sites of NS2/NS3 protease. The information gained from this study will be helpful before synthesizing and testing them. Thus, it can be concluded from this study that cyclic peptides could serve as future drug candidates against DENV as these posses binding affinity against NS2/NS3 protease. The outcome of this study will provide useful information on the drug development and would help in computer aided screening of the drugs against dengue infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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